

***Cylindrocorpus anoplophorae* n. sp. (Nematoda: Cylindrocorporidae) isolated from the white-spotted longicorn beetle, *Anoplophora malasiana* (Coleoptera: Cerambycidae)**

Natsumi Kanzaki<sup>1,2</sup> and Kazuyoshi Futai<sup>1,3</sup>

*Cylindrocorpus anoplophorae* n. sp. is described and figured. The dauer juveniles of *C. anoplophorae* n. sp. were isolated from the genital capsule of the white-spotted longicorn beetle, *Anoplophora malasiana*, and were reared on an Asparagine-Mannitol (A-M) agar. Specimens of adult nematodes for description and measurements were collected from a 2 week-old culture on the A-M agar. The males of the new species have body length of *ca.* 830-1070  $\mu\text{m}$ , the value *a* of *ca.* 19-26, the value *b* of *ca.* 5-6, the value *c* of *ca.* 8-12, *ca.* 29-35  $\mu\text{m}$  length of slightly arcuate spicules, *ca.* 17-20  $\mu\text{m}$  length of thorn shaped gubernaculum, 43-62  $\mu\text{m}$  of tail conoid, nine pares of caudal papillae, and very narrow and tape like leptoderan bursa. The females of new species have body length of *ca.* 980-1260  $\mu\text{m}$ , the value *a* of *ca.* 17-23, the value *b* of *ca.* 6-7, the value *c* of *ca.* 7-9, *ca.* 114-161  $\mu\text{m}$  length of elongated tail, and the values *V* of *ca.* 50-55. The new species is distinguished from the other species within the genus by the morphometric values of male and female, number and arrangement of male caudal papillae, shape and length of male bursa, and tail shape of male and female. Jpn. J. Nematol. 34 (1), 11-19 (2004).

Key words - entomophilic nematode, Japan, morphology, taxonomy.

## INTRODUCTION

Dauer juveniles of a species of "nematode" were isolated from the genital capsule of white-spotted longicorn beetle, *Anoplophora malasiana* Thomson. The juveniles were reared on an Asparagine-Mannitol agar, then the adult nematodes were obtained from the culture, and identified as an undescribed species belonging to the genus *Cylindrocorpus* Goodey, 1939 of family Cylindrocorporidae.

The morphological features of the family

Cylindrocorporidae are defined as follows (Andrassy, 1984; Goodey, 1963): Head with six lips each bearing a papilla of the internal circle, and an external circle of six or 10 papillae which have not described in all genera. Amphids on lateral lips. Stoma with very long protostom. Procorpus and metacorus fused to form a long and cylindrical muscular bulb. Isthmus slender, terminal bulb glandular, but without valve apparatus. Male with single testis, eight to 10 pairs of caudal papillae, spicules paired, gubernaculum present. Bursa present or absent. Female gonad(s) single or paired.

Five genera are included in the family, and each genus is distinguishable from the others based on the male bursa and female gonad(s) (Andrassy, 1984; Poinar et al., 2003): *Cylindrocorpus* has leptoderan bursa and paired

<sup>1</sup> Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

<sup>2</sup> Present: JSPS Post Doctoral Fellow, Laboratory of Forest Protection, Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890-0065, Japan.

<sup>3</sup> Corresponding author, e-mail: futai@kais.kyoto-u.ac.jp

gonads. *Myctolaimus* has no bursa and paired gonads. *Myctolaimellus* has peloderan bursa and paired gonads. *Protocyrrindrocorpus* has lepotoderan bursa and single gonad. *Goodeyus* has peloderan bursa and single gonad.

The genus, *Cylindrocorpus* had established by Goodey (1939). However, Andrassy (1984) deleted the genus *Cylindrocorpus* as a junior synonym of the genus *Myctolaimus* Cobb, 1920, and located following seven species to the genus *Myctolaimus*: *M. curzii* Goodey, 1935; *M. erectus* Massey, 1960; *M. longistoma* (Stefanski, 1922) Andrassy, 1984; *M. macrolaimus* (Schneider, 1866) Massey, 1960; *M. pellucidus* Cobb, 1920; *M. rifflei* Massey and Hinds, 1970; *M. zamithi* (Lordello, 1953) Andrassy, 1984. Poinar et al. (2003), however, reestablished the genus *Cylindrocorpus*, described a species, *C. inevectus*, and moved following four species to the genus *Cylindrocorpus*: *C. curzii* Goodey, 1935; *C. erectus* Massey, 1960; *C. longistoma* Stefanski, 1922; *C. macrolaimus* Schneider, 1866

The newly found nematode is distinguished from all known species in some morphological characters and morphometrical values, and is described herein as *Cylindrocorpus anoplophorae* n. sp.

## MATERIALS AND METHODS

Isolation of nematodes from white-spotted longicorn beetle:

An adult male of *A. malasiana* was obtained at Kitashirakawa Forest Research Station, Field Science Education and Research Center, Kyoto University, on 20 June 2001. The beetle was dissected and examined its nematode infection.

The dauer juveniles of the nematode obtained from the beetle were transferred to Asparagine-Mannitol agar ( $K_2HPO_4$ , 0.1 g;  $MgSO_4 \cdot 7H_2O$ , 0.02 g;  $CaCl_2$ , 0.001 g; NaCl, 0.001 g;  $FeCl_3$ , 0.0002 g;  $KNO_3$ , 0.05 g; asparagine, 0.05 g; mannitol, 0.1 g; agar, 2.0 g; distilled water,

100ml).

Morphological observation:

Adult nematodes were collected from a 2-week-old culture on Asparagine-Mannitol agar using Baermann funnel technique. The nematodes collected were killed by heat (60°C for 5 min.), fixed in TAF (formalin, 7 ml; triethanolamine, 2 ml; distilled water, 91 ml), processed through a glycerol-ethanol series using Seinhorst's method (described in Hooper, 1986) and mounted in glycerin according to Maeseneer and d' Herde's method (described in Hooper, 1986).

### *Cylindrocorpus anoplophorae* n. sp.

(Figs. 1, 2)

Measurements:

See Table 1.

Description:

Male. Cuticle thin, with fine transverse and longitudinal striations. Body cylindrical, curved ventrally when heat-killed. Lip region separated into six protruding lips, each possessing one tiny papilla. Stoma long, narrow, flexible, with thick and distinct cheilorhabdions. Pharyngeal sleeve surrounding 85-90% of total length of stoma. Tooth or denticles absent. Corpus, typical of the family, procorpus and metacarpus fused to form a long and cylindrical muscular bulb, looks roundish rectangle shape. Stoma and corpus longer than isthmus and basal bulb. Isthmus slender, with one-fourth body-diam. width, widening gradually at basal bulb. Basal bulb not muscular, lacking a valve apparatus. Nerve ring encircles the isthmus at the midst of isthmus. Excretory pore between the nerve ring and basal bulb. Cardia well developed, muscular, darkened in color. Testis single, reflexed, sperm in single or double row. Spicules paired, arcuate, distal end pointed, manubrium distinct, knoblike, terminal cucullus absent. Gubernaculum thorn like.

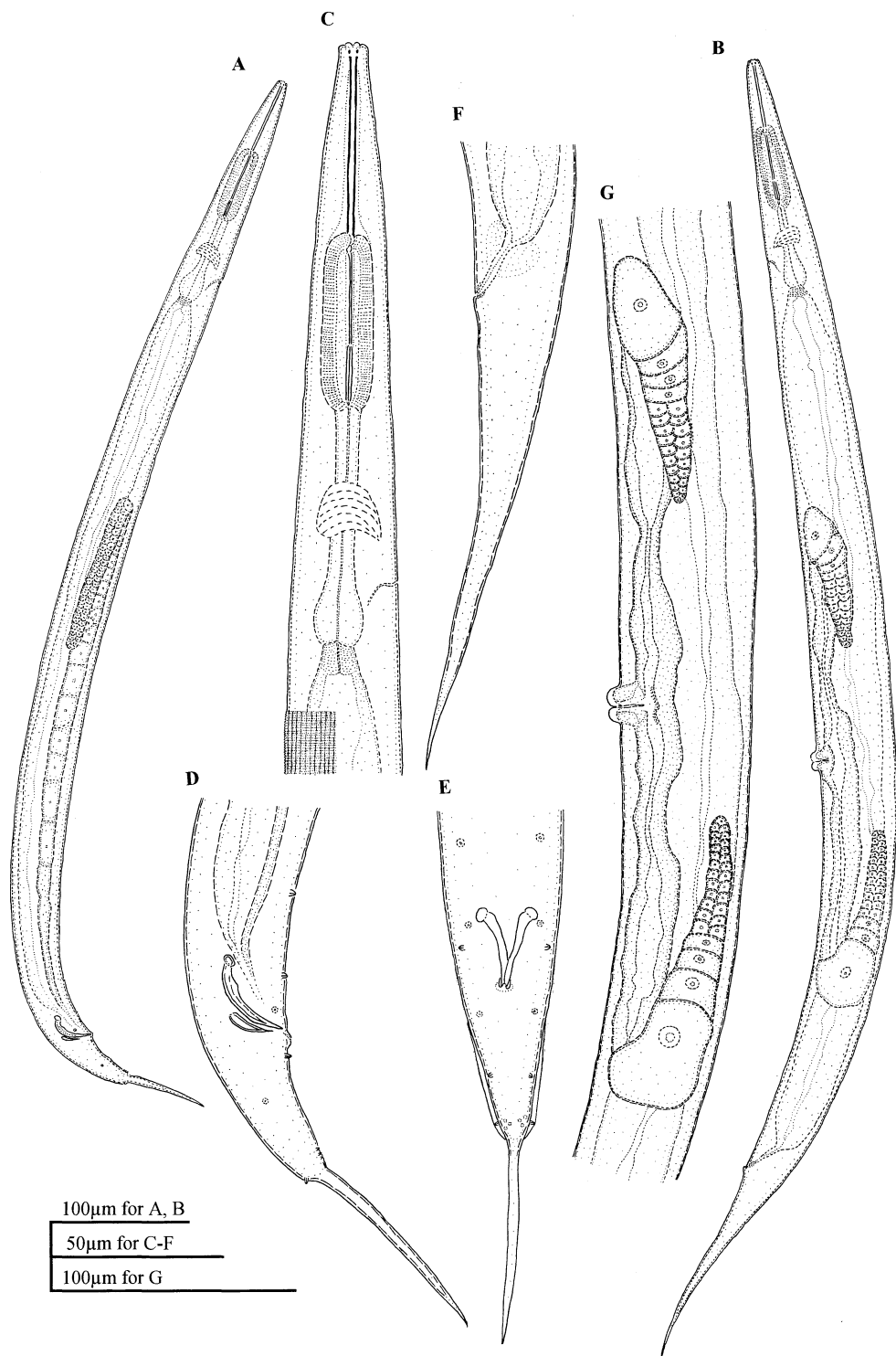


Fig. 1. *Cylindrocorpus anoplophorae* n. sp. Male (A), Female (B), Anterior region of male (C), Lateral view of male tail (D), Ventral view of male tail (E) Female tail (F), Female reproductive organ (G).

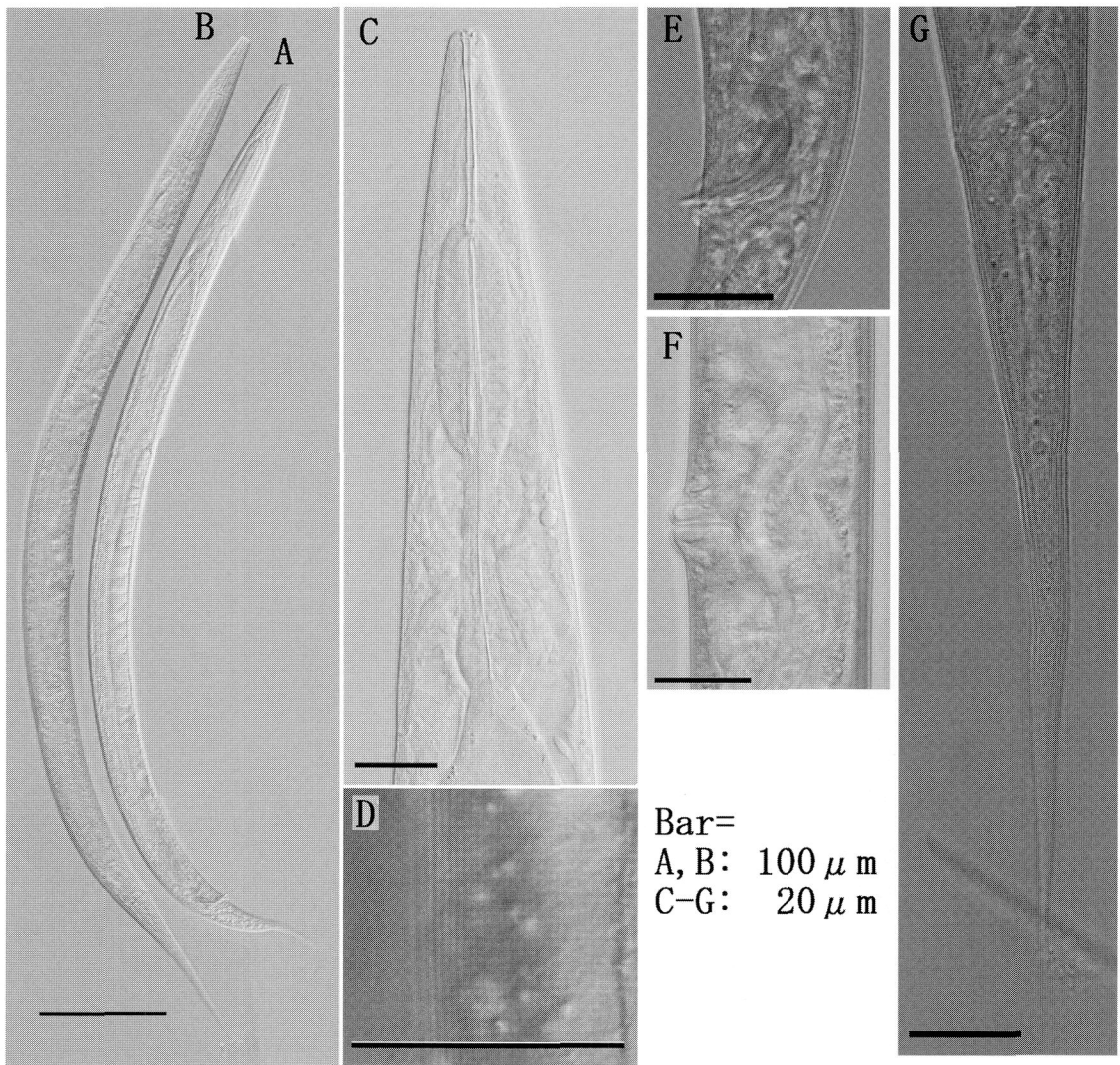


Fig. 2. *Cylindrocorpus anoplophorae* n. sp. Male (A), Female (B), Anterior region of male (C), Body surface of male (D), Lateral view of male tail (E), Female vulva (F), Female tail (G).

Tail ventrally arcuate, with long pointed conoid. Nine pairs of caudal papillae present, first pair anterior to manubrium, second pair at posterior level of manubrium, third pair just anterior to cloacal slit, fourth and fifth pairs at the mid way of anus and the commencement of conoid, the others aggregating at the distal part of tail, third pair at sub ventral, fifth and ninth pairs at lateral, the others at ventral. Bursa leptoderan, very

narrow, tape like, covering between just besides the fourth pair of the caudal papillae and just before conoid.

Female. Body slightly curved when heat-killed. Anterior region similar to that of male. Vulval lip conspicuously protuberant. Vagina strongly muscled, transverse, darkened in color. Reproductive system didelphic. Uterin sac serving as spermatheca, with well developed

Table 1. Measurements of *Cylindrocorpus anoplophorae* n. sp. (all measurements in  $\mu\text{m}$ ).

	Male		Female	
	Holotype	Paratypes <sup>1)</sup>	Allotype	Paratypes <sup>1)</sup>
n	1	15	1	15
L	948	930 $\pm$ 67 (833-1074)	1121	1114 $\pm$ 97 (977-1264)
Body width	44	42 $\pm$ 5 (36-56)	63	59 $\pm$ 6 (50-71)
Stoma length	49	47 $\pm$ 3 (41-54)	49	50 $\pm$ 4 (43-56)
Corpus length	61	60 $\pm$ 3 (54-62)	70	66 $\pm$ 4 (59-70)
Isthmus + basal bulb	61	56 $\pm$ 4 (50-67)	58	59 $\pm$ 4 (53-66)
Pharynx length	122	118 $\pm$ 7 (104-127)	128	121 $\pm$ 7 (111-131)
Anterior end to nerve ring	132	131 $\pm$ 8 (116-145)	150	149 $\pm$ 6 (132-157)
Anterior end to excretory pore	184	161 $\pm$ 10 (152-188)	198	168 $\pm$ 12 (155-198)
Testis length	586	555 $\pm$ 85 (420-706)	-	-
Testis reflection	64	70 $\pm$ 13 (55-100)	-	-
Anterior ovary length	-	-	191	160 $\pm$ 22 (120-191)
Posterior ovary length	-	-	166	161 $\pm$ 23 (120-198)
Anal body width	27	25 $\pm$ 2 (21-29)	31	30 $\pm$ 3 (27-37)
Tail length	114	102 $\pm$ 7 (93-119)	150	137 $\pm$ 12 (114-161)
Tail conoid	62	50 $\pm$ 4.9 (43-62)	-	-
a	21.4	23.2 $\pm$ 1.8 (19.3-26.3)	17.8	19.1 $\pm$ 1.8 (16.7-23.0)
b	5.5	5.7 $\pm$ 0.4 (5.1-6.4)	6.4	6.4 $\pm$ 0.4 (5.7-7.3)
c	8.3	9.2 $\pm$ 0.8 (8.3-11.9)	7.5	8.2 $\pm$ 0.7 (7.0-9.0)
c'	4.2	4.1 $\pm$ 0.2 (3.5-4.7)	4.8	4.5 $\pm$ 0.5 (3.8-5.4)
Spicule <sup>2)</sup>	33	33 $\pm$ 1.8 (29-35)	-	-
Gubernaculum	19	19 $\pm$ 0.8 (17-20)	-	-
T	61.8	58.6 $\pm$ 10.6 (41.3-79.4)	-	-
V	-	-	51.3	52.6 $\pm$ 1.4 (49.8-55.6)

1) Mean  $\pm$  SD (range)

2) Curved median line

sphincter muscle, contains zero to six mature eggs. Ovaries reflex their entire length, oocytes in double rows for approximately half ovary length then in a single row. Anus slightly prominent. Rectum short, half body-diam. long. Tail elongated conical shape tapering to a pointed terminus.

Type habitat and locality:

Type specimens were obtained from 2-week-old culture on Asparagine-Mannitol agar, 26 July 2001. The original culture was started from the dauer juveniles isolated from the male genital capsule of *Anoplophora malasiana* collected at Kitahirakawa Forest Research Station, Field Science Education and Research Center, Kyoto University, Sakyo-ku, Kyoto, 606-8502 Japan.

Type specimens:

Holotype (male) slide number *Cylindrocorpus anoplophorae* M-01 (USDA Nematode Collection deposition number: *Cylindrocorpus anoplophorae* T- 573t); allotype (female) slide number *Cylindrocorpus anoplophorae* F-01 (USDA Nematode Collection deposition number: *Cylindrocorpus anoplophorae* T- 574t), USDA Nematode Collection, Beltsville, MD, USA. Paratypes (15 females and 15 males): slide numbers *Cylindrocorpus anoplophorae* M-2-10 (USDA Nematode Collection deposition number: *Cylindrocorpus anoplophorae* T- 5155p - T- 5163p) (nine males), F-2-10 (USDA Nematode Collection deposition number: *Cylindrocorpus anoplophorae* T- 5164p- T-5172p) (nine females) deposited in USDA Nematode Collection, Beltsville, MD, USA; slide numbers *Cylindrocorpus anoplophorae* M-11-15 (five males), F-11-15 (five females), The Herbarium and Insect Museum of the National Institute of Agro-Environmental Science, Tsukuba, Ibaraki, Japan; slide number *Cylindrocorpus anoplophorae* M-16 (one male), F-16 (one female), Kyoto University, Environmental Mycology Laboratory Collection, Kyoto, Japan.

Diagnosis and relationships:

*Cylindrocorpus anoplophorae* n. sp. is characterised by very narrow, short, and leptoderan bursa covering between the fourth papillae level and proximal part of the conoid, nine pairs of caudal papillae (3 preanal and 6 postanal), long spicule, and long tail conoid of males, and long tail of females.

Five species have been included in the genus *Cylindrocorpus*: *C. curzii*, *C. erectus*, *C. inevectus*, *C. longistoma*, and *C. macrolaimus* (Poinar et al., 2003). *C. anoplophorae* n. sp. is distinguished from *C. curzii*, *C. erectus*, *C. inevectus*, and *C. longistoma* by the number and arrangement of male caudal papillae and tail conoid of males (Fig. 1 D, E, Table 2). Males of *C. anoplophorae* n. sp. have nine pairs of caudal papillae, i.e., three pairs at pre anal and six pairs at post anal, and long tail conoid, while the males of *C. curzii* have 10 pairs of caudal papillae (3 pre anal and 7 post anal) and short tail conoid (Goodey, 1935), *C. longistoma* have 10 pairs of caudal papillae (3 pre anal and 7 post anal) and long tail conoid (Goodey, 1927; Stefanski, 1922), and the males of *C. erectus* and *C. inevectus* have eight pairs of them (2 pre anal and 6 post anal) and short tail conoid (Massey, 1960; Poinar et al., 2003).

The morphometric values are also available for diagnostics for *Cylindrocorpus* species (see Table 2). The body lengths of *C. anoplophorae* n. sp. are larger than those of *C. erectus* (Massey, 1960) and *C. inevectus* (Poinar et al., 2003). The values *a* of *C. anoplophorae* n. sp. are smaller than those of *C. curzii* (Goodey, 1935) and *C. longistoma* (Stefanski, 1922), and larger than those of *C. erectus* (Massey, 1960). The spicule and gubernaculum lengths of *C. anoplophorae* n. sp. are larger than those of *C. curzii* (Goodey, 1935), *C. inevectus* (Poinar et al., 2003), and *C. longistoma* (Stefanski, 1922). The value *V* of *C. anoplophorae* n. sp. is larger than those of *C.*

Table 2. Morphometric comparisons of *Cylindrocorpus* species (all measurements in  $\mu\text{m}$ ).

	<i>C. anoplophorae</i> n. sp.		<i>C. curzii</i> <sup>1)</sup>		<i>C. erectus</i> <sup>2)</sup>		<i>C. inevectus</i> <sup>3)</sup>		<i>C. longistoma</i> <sup>4)</sup>	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
n	16	16	? <sup>5)</sup>	? <sup>5)</sup>	1	1	20	20	? <sup>5)</sup>	? <sup>5)</sup>
L	833-1074	977-1264	720-1080	878-1430	770	950	634-800	703-985	860-950	1000-1034
a	19.3-26.3	16.7-23.0	23-29	19-30	17	16	19-24	23-31	35-37	29-33
b	5.1-6.4	5.7-7.3	5.1-6.6	6.4-7.8	7.7	5.5	7.3-9.2	8.9-10.6	7.0-7.5	6.0-6.4
c	8.3-11.9	7.0-9.0	25-32	6.4-8.8	19.8	8.8	20-25	5.8-9.9	4.7-4.8	4.0-4.2
c'	3.5-4.7	3.8-5.4	* <sup>6)</sup>	* <sup>6)</sup>	* <sup>6)</sup>	5-6 <sup>6)</sup>	* <sup>6)</sup>	* <sup>6)</sup>	* <sup>6)</sup>	* <sup>6)</sup>
Tail length	93-119	114-161	45 <sup>7)</sup>	* <sup>6)</sup>	30 <sup>7)</sup>	100 <sup>7)</sup>	28-44	72-170	180 <sup>7)</sup>	300 <sup>7)</sup>
Tail conoid	43-62	-	15 <sup>7)</sup>	-	8 <sup>7)</sup>	-	2-10	-	150 <sup>7)</sup>	-
Spicule	29-35	-	23-26	-	* <sup>2)</sup>	-	19-28	-	22-26	-
Gubernaculum	17-20	-	12-14	-	* <sup>2)</sup>	-	7-15	-	12-13	-
V	-	49.8-55.6	-	48-53	-	47	-	47-55	-	45-47

Morphometrics are not given for *C. macrolaimus* (Schneider, 1866).

1) After Goodey (1935). 2) After Massey (1960). 3) After Poinar et al. (2003). 4) After Stefanski (1922) and Goodey (1927).

5) ? : Sample numbers are not given. 6) \* : Values are not given. 7) Calculated from the original figure.

*erectus* (Massey, 1960) and *C. longistoma* (Stefanski, 1922).

*Cylindrocorpus anoplophorae* n. sp. could not be distinguished from *C. macrolaimus* by the number and arrangement of male caudal papillae and the shape of male tail conoid. Thus the new species seems morphologically closest to *C. macrolaimus*. The morphometric values of *C. macrolaimus* are not given, and detailed morphological traits are not described, however, *C. anoplophorae* n. sp. can be distinguished from *C. macrolaimus* based on the shape of male bursa. Males of *C. anoplophorae* n. sp. have very narrow, tape like, and short bursa, which elongates between just besides the fourth pair of the caudal papillae and just before conoid (Fig. 1E), while the bursa of *C. macrolaimus* is flap like and elongating between just besides the second pair of the caudal papillae and just before conoid (Schneider, 1866).

## REMARKS

The *Cylindrocorpus* species have been reported as the saprophagous or entomophilic free-living nematodes and have variable habitats. As for the saprophagous species, *C. curzii*

was found in a rotting yam from Nigeria and rotting tissue of bitter cassava in Italian Somaliland (Goodey, 1935; Goodey, 1963), *C. longistoma* was reported from rat faeces in UK and ginger roots, imported from China (Goodey, 1927; Goodey, 1963; Stefanski, 1922), and *C. macrolaimus* was found in moist soil and decaying matter (Schneider, 1866; Goodey, 1963). While for the entomophilic species, *C. erectus* was reported as "associated with *Scolytus multistriatus* in American elm, *Ulmus americana*" in the United States (Massey, 1960; 1974), and *C. inevectus* was described from mature and decaying oil palm flowers, *Elaeis guineensis* and from the under surface of the elytra of the oil palm weevil, *Elaeidobius kamerunicus* (Poinar et al., 2003).

The dauer juveniles of *C. anoplophorae* n. sp. were isolated from the male genital capsule of white-spotted longicorn beetle. Thus the new species is assumed to inhabit in the xylem of woody plants, the host trees of the beetle.

We concluded that *C. anoplophorae* n. sp. is close to *C. macrolaimus* based on the morphological traits, i.e., the number and the arrangement of caudal papillae and tail conoid of males.

However, *C. macrolaimus* is described as the saprophagous soil nematode (Schneider, 1866), whereas, *C. anoplophorae* n. sp. was isolated from a species of wood inhabiting beetle, *A. malasiana*. Therefore the biological features of *C. anoplophorae* n. sp. might be rather close to *C. erectus* and *C. inevectus*, the associate of bark beetle (Massey, 1960), and that of the oil palm weevil (Poinar et al., 2003), respectively. The life history of *Cylindrocorpus* species cannot be generalized by above descriptions, because the habitats differ significantly among species and information about vectors are not given for *C. longistoma* and *C. macrolaimus*. Therefore the examinations on the life history of each species are needed to understand the biological characteristics and the systematic relationship of the genus.

#### LITERATURE CITED

- Andrassy, I. (1984) Klasse Nematoda. Gustav Fischer Verlag, Stuttgart, 509pp.
- Cobb, N. A. (1920) One hundred new nemas. Contributions to a Science of Nematology 9, 217-343.
- Goodey, J. B. (1963) Soil and Freshwater Nematodes (Rev. ed.). Methuen & Co. Ltd. London, 544pp.
- Goodey, T. (1927) *Cylindrogaster coprophaga* gen. et sp. nov. a nematode found in a culture of faeces from a wild brown rat. Journal of Helminthology 5, 25-32.
- Goodey, T. (1935) On *Cylindrogaster curzii* n. sp., a saprophagous nematode. Journal of Helminthology 13, 19-24.
- Goodey, T. (1939) *Cylindrocorpus* nom. nov. for *Cylindrogaster* Goodey, 1927 (Nematoda). Journal of Helminthology 17, 149-150.
- Hooper, D. J. (1986) Handling, fixing, staining and mounting nematodes. In: Laboratory methods for work with plant and soil nematodes. (6<sup>th</sup>. Ed.) (Southey, J. F., ed.) Her Majesty's Stationary Office, London, pp. 59-80.
- Lordello, L. G. E. (1953) Contribuição ao conhecimento dos nematódeos do solo de algumas regiões do Estado de São Paulo. Doctoral Thesis, Piracicaba. 75pp.
- Massey, C. L. (1960) A new species of nematoda, *Cylindrocorpus erectus* associated with *Scolytus multistriatus* Marsh in American elm. Proceedings of the Helminthological Society of Washington 27, 42-44.
- Massey, C. L. (1974) Biology and taxonomy of nematode parasites and associates of bark beetle in the United States. USDA Agriculture Handbook No. 446, 233pp.
- Massey, C. L. and Hinds, T. E. (1970) Nematodes from aspen canker in Colorado and New Mexico. Canadian Journal of Zoology 48, 97-108.
- Poinar, G. O., Jackson, T. A., Bell, N. L. and Wahid, M. B. (2003) *Cylindrocorpus inevectus* sp. n. associated with the oil palm weevil, *Elaeidobius kamerunicus* (Faust) (Coleoptera: Curculionidae), with a synopsis of the family Cylindrocorporidae and establishment of Longibuccidae n. fam. (Diplogastroidea: Nematoda). Nematology 5, 183-190.
- Schneider, A. F. (1866) Monographie der Nematoden. G. Reimer, Berlin, 357pp.
- Stefanski, W. (1922) Excrétion chez les nématodes libres. Archiwum Nauk Biologicznych Towarzystwa Naukowego Warszawskiego 1, 1-33.

Received October 23, 2003.



## 和文摘要

## ゴマダラカミキリ

*Anoplophora malasiana* から

検出された線虫

*Cylindrocorpus anoplophorae* n. sp.

神崎 菜摘・二井 一禎

*Cylindrocorpus* 属の未記載種を *C. anoplophorae* n. sp. として記載した。本種の耐久型幼虫は、ゴマダラカミキリ、*Anoplophora malasiana* の雄成虫の生殖器から検出された。記載にはアスパラ

ギン-マンニトール培地上で2週間生育したカルチャーから得られた雌雄成虫を用いた。本種雄成虫は、体長約830-1070  $\mu\text{m}$ 、a 値約19-26、b 値約5-6、c 値約8-10であり、長さ約28-35  $\mu\text{m}$  のやや湾曲した交接刺、長さ約17-20  $\mu\text{m}$  の爪状の副刺、長さ約43-62  $\mu\text{m}$  の尾端突起、9対の尾乳頭、非常に幅の狭い尾翼を有していた。雌成虫は、体長約980-1260  $\mu\text{m}$ 、尾長約114-161  $\mu\text{m}$ 、a 値約17-23、b 値約6-7、c 値約7-9、V 値約50-56であった。本種は、雌雄成虫の形態計測値、尾部の形状、雄成虫の尾翼の形態、尾乳頭の配列により、本属既知種と識別可能であった。